

Amendments to the Specifications:

Please replace the paragraph starting at page 17, line 4 of the specification with the following paragraph:

In one aspect of the present invention the synthetic peptide for antibody and cell line generation as described above is (Xaa)_m-His-Arg-Gly-Tyr:NO₂-Pro-Gly-(Xaa)_n (SEQ ID NO: 3), wherein Xaa denote any amino acid or derivatives thereof and m and n are independent integers e.g. from 0 to 10.

Please replace the paragraph starting at page 17, line 10 of the specification with the following paragraph:

In one preferred embodiment of the present invention the synthetic peptide for antibody and cell line generation as described above is (Xaa)_m-His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly-(Xaa)_n (SEQ ID NO: 4), wherein Xaa denote any amino acid or derivatives thereof and m and n are independent integers e.g. from 0 to 10.

Please replace the paragraph starting at page 17, line 16 of the specification with the following paragraph:

In another preferred embodiment the synthetic peptides for antibody and cell line generation as described above has the form (Xaa)_m-Leu-Gln-Tyr:NO₂-Met-Arg-Ala-(Xaa)_n (SEQ ID NO: 5), wherein Xaa denote any amino acid or derivatives thereof and m and n are independent integers e.g. from 0 to 10.

Please replace the paragraph starting at page 19, line 4 of the specification with the following paragraph:

FIG. 2 shows competitive inhibition of antiserum D37 binding to His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6) coated plates using His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 1)(SEQ ID NO: 6)(O), His-Arg-Gly-Tyr-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 1)(●), native type II collagen (◆), nitrated type II collagen (◇), type I collagen (), BSA () and nitrated BSA (∇) as competitors. B/Bo represents the ratio between antibody bound to coated antigen in the presence of competitor antigen (B) or in the absence of competitor antigen (Bo) and is given in percentage;

Please replace the paragraph starting at page 20 line 14 of the specification with the following paragraph:

A sequence of nine amino acids (His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly) (SEQ ID NO: 6) derived from the triple helical region of type II collagen [(α1) II] and a second sequence of six amino acids Leu-Gln-Tyr:NO₂-Met-Arg-Ala (SEQ ID NO: 7) derived from the C-telopeptide of type II collagen were synthesized using standard Fmoc solid-phase peptide synthesis (HBTU/HOBt protocol) (Chan, W. C. and White, P. D., 2000).

Please replace the paragraph starting at page 21, line 3 of the specification with the following paragraph:

The following examples will concentrate on antisera achieved from immunisation with the His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6) peptide. All examples can be performed in similar ways for the Leu-Gln-Tyr:NO₂-Met-Arg-Ala (SEQ ID NO: 7) peptide.

Please replace the paragraph starting at page 21, line 8 of the specification with the following paragraph:

Six antisera, identified as Coll2-1:NO2 D35, D36, D37, D38 D39 and D40, were obtained and their specificity were tested with the competitive inhibitions His-Arg-Gly-Tyr(NO₂)-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6), His-Arg-Gly-Tyr-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 1), type II nitrated collagen, native type II collagen, type I nitrated collagen I, type I collagen, nitrated BSA and BSA.

Please replace the paragraph starting at page 21, line 16 of the specification with the following paragraph:

A competitive immunoassay was developed to quantify breakdown products of nitrated type II collagen containing following sequence His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6). Synthetic His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6) peptides were biotinylated and incubated at 1.25 ng/ml on streptavidin coated plates (Nunc, Denmark) for 1 hour at room temperature. Fifty µl of calibrators (to generate a standard curve) or unknown samples, diluted in Ultrosor G (Gibco) were added to separate wells. Hundred µl antiserum (see above) diluted {fraction (1/125000)} was added to each well. Samples were mixed by rotating the plate and incubated 1 hour at room temperature. After three successive washings with washing buffer (Tris 25 mM, NaCl 50 mM pH 7.3), 100 µl of horseradish peroxidase-conjugated goat antibodies to rabbit IgG (Biosource, Belgium) were added to each well and incubated 1 hour at room temperature. After washing, 100 µl of freshly prepared enzyme substrate (TMB, Biosource, Belgium) were added to each well. After 15 minutes incubation, the reaction was stopped with 100 µl 4M H₃PO₄. The coloration was read with a microplate reader (Labsystem iEMS Reader MF, Finland) at 450 nm and corrected for absorbance at 620 nm. A standard curve was constructed on a log-linear graph by plotting the B/Bo of 6 calibrators (10 to 0.01 nM) (FIG. 1). The concentration of HIS-ARG-GLY-TYR:NO₂-PRO-GLY-LEU-ASP-GLY (SEQ ID NO: 6) containing peptides in the samples, were determined by interpolation on the calibration curve.

Please replace the paragraph starting at page 22, line 22 of the specification with the following paragraph:

The antisera produced, were tested for their specificity for His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6), by use of the immunoassay described in example 1. To test for specificity His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6), His-Arg-Gly-Tyr-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 1) peptide, type II nitrated collagen, native type II collagen, type I nitrated collagen, type I collagen, nitrated BSA and BSA.

Please replace the paragraph starting at page 22, line 29 of the specification with the following paragraph:

Native type II collagen, type I collagen, nitrated collagen type I, nitrated BSA and BSA, were not able to compete with the coated His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6) peptide in the applied concentrations, whereas the antiserum showed weak affinity to the non-Nitrated His-Arg-Gly-Tyr-Pro-Gly-Leu-Asp-Gly sequence (SEQ ID NO: 1) and nitrated collagen type II and strong affinity to the His-Arg-Gly-Tyr:NO₂-Pro-Gly-Leu-Asp-Gly (SEQ ID NO: 6) sequence. A lack of binding affinity has also been demonstrated with L-nitrotyrosine.

Please replace the paragraph starting at page 29, line 12 of the specification with the following paragraph:

Rabbits were injected intra-peritoneally with 1 ml of the conjugated peptides (0.5 mg/ml) emulsified in complete Freund's adjuvant. The conjugate and the adjuvant were mixed in equal volumes. Injections were repeated four times every month using the same peptide concentration that those of the first injection in incomplete Freund's adjuvant. Ten days after the last injection, the rabbits were sacrificed. Blood was collected and centrifuged for 10 min at 2500 rpm at 4°C. The supernatant was kept and stored at -20°C. At each bleeding, antisera were screened by titration

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experiment for the presence of anti-GGGLQY(NO₂)MRA (SEQ ID NO: 8) antibody. The antisera
with the highest titers were selected for the following experiments.